

# TranSignal™ PDZ Domain Arrays

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Product User Manual  
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## **2 | TranSignal™ PDZ Domain Arrays**

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## 1. ARRAY OVERVIEW

Panomics' TranSignal™ PDZ Domain Arrays include a total of 123 different human PDZ domains. These arrays are made using the recombinant conserved binding sites of individual PDZ domain proteins fused with glutathione-S-transferase (GST). Proteins are affinity-purified and immobilized onto a membrane. Each PDZ domain on the arrays is spotted in duplicate.

For a complete map and list of the domains included on the arrays, see Appendices A, B, C, and D.

## 2. INTRODUCTION & BACKGROUND

### PDZ Domain Structure & Function

A key to understanding cellular signal transduction is clarifying how proteins interact with one another. Protein-protein interactions are often mediated by noncatalytic, conserved domains. One of these domains is the PDZ domain.

PDZ domains are regions of sequence homology found in diverse signaling proteins (1–3). The name "PDZ" derives from the first three proteins in which these domains were identified: PSD-95, a protein involved in signaling at the post-synaptic density; DLG, the *Drosophila* Discs Large protein; and ZO-1, the zonula occludens 1 protein (1–3). PDZ domains are also sometimes called DH domains or GLGF repeats.

PDZ domains act as modules for protein-protein interactions. PDZ domains recognize specific motifs that occur at the C-termini of target proteins; these motifs are most often present in the cytoplasmic tails of transmembrane receptors and channels (4, 5). PDZ domains can also recognize internal sequences (6–9).

By recruiting downstream proteins in a signaling pathway, PDZ domains mediate assembly of specific multi-protein complexes. Proteins that contain PDZ domains play important roles in many key signaling pathways, including maintaining epithelial cell polarity and morphology (10, 11), organizing the postsynaptic density in neuronal cells, and regulating the activity and trafficking of membrane proteins (12).

## **4** | TranSignal™ PDZ Domain Arrays

### **Valuable Tool for Studying PDZ Domains**

Now that the draft sequence of the human genome is complete, we are faced with myriad new proteins whose functions remain a mystery. An important step toward characterizing the function of a PDZ domain-interacting ligand and/or protein is to identify to which PDZ domain it binds, and hence determine with which signaling pathway it is involved. Traditional methods for detecting protein-protein interactions, such as co-immunoprecipitation, are arduous and time consuming at best. Panomics offers a convenient tool for identifying interactions between a ligand and PDZ domains that remain folded in active conformations.

With Panomics' TranSignal PDZ Domain Arrays, you can determine whether your protein of interest binds to multiple PDZ domains—all in one experiment. The assay couldn't be simpler: just express your protein of interest in bacteria and incubate the extract with the TranSignal PDZ Domain Array membrane. The protein interactions literally take place on the array membrane, and you can visualize them using HRP-based chemiluminescence detection.

The TranSignal PDZ Domain Arrays can also be used when the ligand of interest is available as a biotinylated synthetic peptide (see Appendix D) or purified protein when the appropriate secondary antibody for detection is available. Biotin uses anti-streptavidin HRP conjugate and purified protein uses an antibody specific to the protein followed by a corresponding HRP-conjugate.

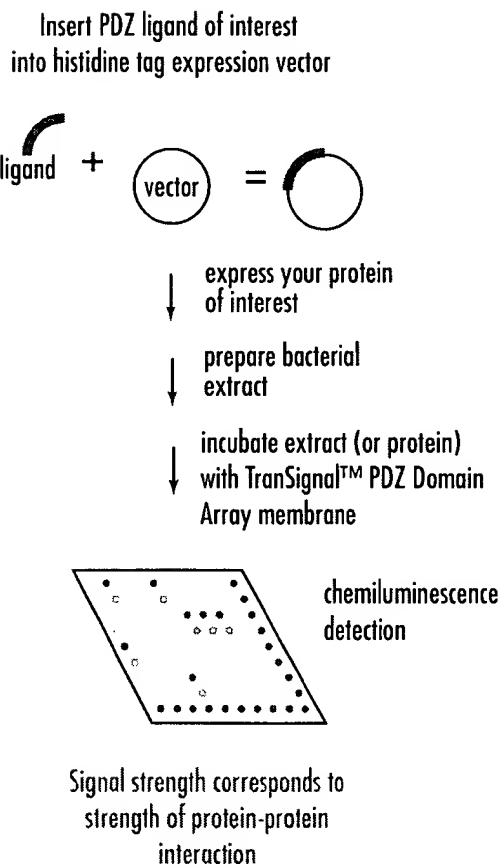


Figure 1: Flow chart of the TransSignal™ PDZ Domain Array assay.

## **6 | TranSignal™ PDZ Domain Arrays**

### **3. MATERIALS PROVIDED**

**STORAGE CONDITIONS:** Upon receipt, the array membranes; Anti-Histidine HRP Conjugate; Blocking and Wash Buffers; and Detection Buffers A & B should be kept at 4°C until use. The PDZ Control Bacterial Extract should be stored at -20°C.

- **TranSignal PDZ Domain Array** (2 each; 3 cm wide x 6 cm long)
- **20X PDZ Control Bacterial Extract** (500 µl)  
(From *E. coli* expressing PDZ ligand)
- **400X Anti-Histidine HRP Conjugate** (20 µl)
- **1X Blocking Buffer** (15 ml)
- **1X Resuspension Buffer** (60 ml)
- **10X Wash Buffer** (15 ml)—dilute to 1X with dH<sub>2</sub>O
- **Detection Buffer A** (600 µl)
- **Detection Buffer B** (600 µl)
- **Four-Well Tray**

Sufficient quantities of each buffer are provided for two assays.

### **4. ADDITIONAL MATERIALS REQUIRED**

#### **3.1 Reagents and Solutions**

- **Histidine Tag Expression Vector** (pET Systems from Novagen or pQE Systems from Qiagen)
- **DH5α Competent Cells** (Gibco BRL, Cat. # 18265-017)
- **LB Amp-100 Broth** (Teknova, Cat. # L8100); 1.0% tryptone, 0.5% yeast extract, 1.0% NaCl with 100 µg/ml ampicillin
- **IPTG** (Teknova, Cat. # I3425); isopropyl β-D-thiogalactopyranoside

#### **3.2 Materials and Equipment**

- **Microcentrifuge**
- **Sonicator**
- **Orbital shaker**
- **Hyperfilm™ ECL** (Amersham, Cat.# RPN1674K) or equivalent  
OR
- **Chemiluminescence imaging system** (e.g., FluorChem™ from Alpha Innotech Corp.)

## 5. PREPARATION OF BACTERIAL EXPRESSION CONSTRUCT

Insert the PDZ ligand of interest into any commercially available histidine tag expression vector using standard molecular cloning techniques (13). Transform into DH5 $\alpha$  competent cells with DNA ligation mix as described by the manufacturer's instructions, and screen for ligand-harboring bacteria using standard molecular techniques (13). After screening, transform the recombinant clone into the manufacturer's recommended strain for expression of fusion protein, e.g., BL21(DE3). We recommend checking that the transformed bacteria express ligand fusion protein by SDS-PAGE and/or Western blot analysis.

## 6. PREPARATION OF BACTERIAL EXTRACTS WITH PDZ LIGAND

In this section, you will prepare bacterial extract containing your ligand of interest for hybridization with the array membrane (Section 7). For best results, we recommend that the protein concentration of your extract be at least 0.1–1 mg/ml.

- 6.1 Inoculate the transformed bacteria in 1 ml of LB/Amp (100  $\mu$ g/ml) (Section 5).
- 6.2 Grow bacteria overnight at 37°C with shaking at 250 rpm.
- 6.3 Transfer 80  $\mu$ l of the overnight culture to a tube containing 4 ml of LB/Amp (100  $\mu$ g/ml).
- 6.4 Grow bacteria at 37°C until OD600 readings are approx. 0.5–0.8.
- 6.5 Add 100  $\mu$ M–1mM IPTG.
- 6.6 Continue to grow for an additional 3–4 hr at 37°C.
- 6.7 Collect cells by centrifugation. Decant supernatant.
- 6.8 Resuspend the pellet in 750  $\mu$ l of ice-cold 1X Resuspension Buffer.
- 6.9 Lyse cells using a sonicator.
- 6.10 Centrifuge at 14,000 rpm for 5 min at 4°C.
- 6.11 Transfer supernatant into a clean microcentrifuge tube.
- 6.12 Store on ice until further use. For longer storage, keep at –20°C.

## **8 | TranSignal™ PDZ Domain Arrays**

### **7. INCUBATION**

In this Section, you will incubate the bacterial extract containing your PDZ ligand of interest (prepared in Section 6) to the array membrane. Note that the array membranes have a notch at the top right-hand corner for orientation purposes.

*Note: Be sure that the membrane is fully submerged in assay buffer at all times. Never let the membrane dry out.*

- 7.1 Place each membrane into the provided four-well tray containing 5 ml of 1X Blocking Buffer. Make sure that the membrane is fully submerged in buffer.
- 7.2 Place the tray on a shaker and incubate for 1 hr at room temperature or overnight at 4°C.
- 7.3 Remove 1X Blocking Buffer, and briefly rinse membrane with 6 ml of 1X Wash Buffer.
- 7.4 Incubate the membrane with the diluted bacterial extract (or use the provided 20X Positive Control Extract diluted to 1X in 1X Resuspension Buffer) with gentle shaking for 1-2 hr at room temperature or overnight at 4°C.
- 7.5 After incubation, wash the membrane three times with 6 ml of 1X Wash Buffer for 10 min (each wash) at room temperature.
- 7.6 Incubate the membrane with 4 ml of 1X Anti-Hisitidine HRP Conjugate (diluted from 20X in 1X Wash Buffer) for 1-2 hr at room temperature.
- 7.7 Wash three times with 6 ml of 1X Wash Buffer for 10 min (each wash) at room temperature.

## 8. DETECTION

**Important note:** Do not let the membrane dry out during detection.

- 8.1 Prepare the detection solution immediately before use by mixing equal amounts of Detection Buffers A and B—e.g., 300 µl of Detection Buffer A and 300 µl of Detection Buffer B.
- 8.2 Using forceps to hold the cut corner, carefully remove each membrane from its tray. Drain the excess Wash Buffer from the membrane by touching the edge against tissue. Place protein- side up by orienting the notch to the top, right-hand corner on a clean plastic sheet.
- 8.3 Pipet the mixed Detection Buffers onto the membrane. Ensure that the buffer mixture is evenly distributed over the membrane without air bubbles.
- 8.4 Incubate for 5 min at room temperature.
- 8.5 Remove excess substrate by holding the membranes with forceps and touching the edge against tissue. Place the membrane between two plastic sheets and gently press on the top sheet to remove air bubbles.
- 8.6 Expose the membranes using either Hyperfilm™ ECL or a chemiluminescence imaging system, such as the FluorChem™ imager from Alpha Innotech Corp. In either case, we recommend that you try several different exposures of varying lengths of time (e.g., 30 sec–5 min).

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### 9. TROUBLESHOOTING GUIDE

| Problem   | Cause   | Recommendation   |
|---|---|--|
| Weak or no signal                                 | Expressed ligand does not have a Histidine-tag.                               | Check construct by DNA sequencing. Ensure that the cloned insert does not contain an internal translational start site.  |
|   | Histidine-tag is partially hidden.  | Protein binding may be hindered by a partially hidden Histidine-tag. Try using a high concentration (5-10X of the bacterial lysate) or longer binding time                     |
| High background                                   | Concentration of bacterial lysate is too high.                                | Further dilute bacterial lysate (e.g. 30X).  |
|   | Antibody concentration is too high.   | Further dilute the antibody.   |
|   | Nonspecific interactions with antibodies or other reagents used in the assay. | Check signal using a zero standard (i.e. PVDF membrane alone). High background is usually the result of insufficient blocking. Try longer incubation with the blocking buffer. |
| Uneven background                                 | Membrane dried out during incubation.   | Dilute the Detection Buffer.   |
|   |   | Keep the membrane fully submerged in solution during all incubation steps.   |
|   | Volume of blocking solution, bacterial lysate or antibody is too low.         | Increase the volume to make sure that the membrane is fully submerged during incubation.   |
|   | Volume of detection buffer is too low.  | Increase the volume to make sure that the membrane surface is fully covered.   |
| Air bubbles on membrane surface during detection. |   | Remove air bubbles from membrane surface.  |
|   |   |  |

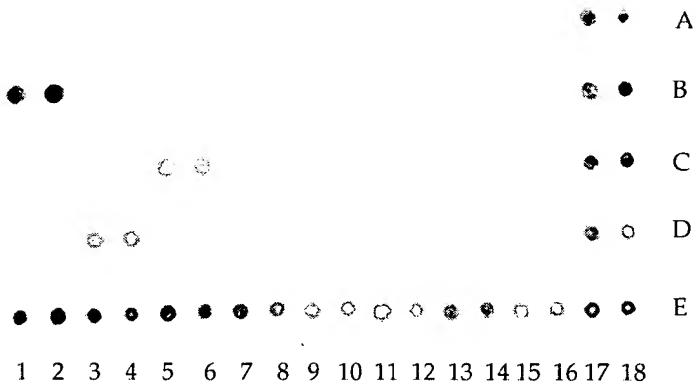
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## 12 | TranSignal™ PDZ Domain Arrays

### APPENDIX A: Typical Results, Schematic Diagram & Domain List for PDZ Domain Array I



*Typical results obtained with the TranSignal PDZ Domain Array I. Class Kv1.4 PDZ ligand from bacterial extract specifically interacts with corresponding PDZ domains. Kv1.4 ligand was expressed as histidine tag fusion protein. Bacterial extract was hybridized with the TranSignal PDZ Domain Array, and the image was acquired using FluorChem™ imager (from Alpha Innotech). Spots with stronger intensities indicate higher binding affinity of the ligand of interest to PDZ Domain(s).*

|   | 1         | 2         | 3         | 4         | 5         | 6       | 7         | 8         | 9   | 10  | 11  | 12  | 13  | 14  | 15  | 16  | 17  | 18 |
|---|-----------|-----------|-----------|-----------|-----------|---------|-----------|-----------|-----|-----|-----|-----|-----|-----|-----|-----|-----|----|
| A | Mint-2-D1 | Mint-3-D1 | Mint-3-D2 | Mint-1-D1 | Mint-1-D2 | CSPK    | Dlg-D1    | Dlg-D3    |     |     |     |     |     |     |     |     | pos |    |
| B | Dlg2-D2   | Dlg4-D3   | DVL1      | DVL3      | DVLL      | GIPC    | HtrA2     | LIMK2     |     |     |     |     |     |     |     |     | pos |    |
| C | MPP2      | NEB1      | OMP25     | hCLIM1    | PTPH1     | ZO-2-D1 | hPTP1E-D1 | hPTP1E-D6 |     |     |     |     |     |     |     |     | pos |    |
| D | RGS12     | RIL       | ZO-1-D3   | ZO-2-D3   | GST       |         |           |           |     |     |     |     |     |     |     |     | pos |    |
| E | pos       | pos       | pos       | pos       | pos       | pos     | pos       | pos       | pos | pos | pos | pos | pos | pos | pos | pos | pos |    |

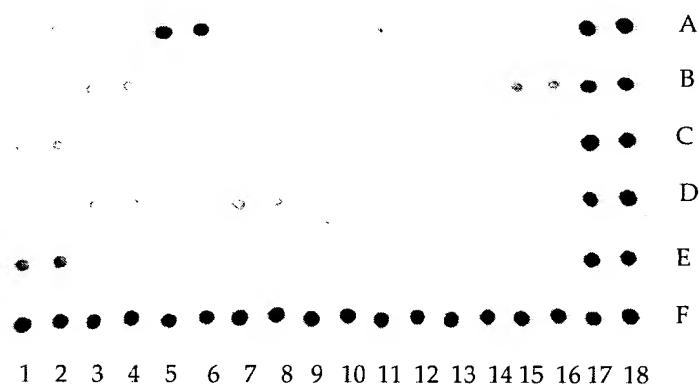
*Schematic diagram of the TranSignal PDZ Domain Array I. The proteins on the array are spotted in duplicate at 100 ng. Histidine-tagged ligand has been spotted along the bottom (row F) and in duplicate along the right side (column 17, 18) of the membrane. These spots are intended for alignment. Note that the notch is at the top right-hand corner.*

## PDZ domain list • TranSignal™ PDZ Domain Array I

| POSITION | PDZ DOMAIN | FULL NAME  |
|----------|------------|--|
| A1, 2    | Mint-2-D1  | X11L protein, PDZ domain #1  |
| A3, 4    | Mint-3-D1  | X11L2 protein, PDZ domain #1   |
| A5, 6    | Mint-3-D2  | X11L2 protein, PDZ domain #2   |
| A7, 8    | Mint-1-D1  | X11 protein, PDZ domain #1   |
| A9, 10   | Mint-1-D2  | X11 protein, PDZ domain #2   |
| A11, 12  | CSKP       | Calcium/calmodulin-dependent serine protein kinase                     |
| A13, 14  | Dlg-D1     | Synapse-associated protein 97(SAP-97), PDZ domain #1                   |
| A15, 16  | Dlg1-D3    | Synapse-associated protein 97(SAP-97), PDZ domain #3                   |
| B1, 2    | Dlg2-D2    | Channel associated protein of synapse-110 (Chapsyn-110), PDZ domain #2 |
| B3, 4    | Dlg4-D3    | Human postsynaptic density-95 (PSD-95), PDZ domain #3                  |
| B5, 6    | DVL1       | Dishevelled 1  |
| B7, 8    | DVL3       | Dishevelled 3  |
| B9, 10   | DVLL       | Dishevelled-1-like   |
| B11, 12  | GIPC       | GAIP C-terminus interacting protein GIPC                               |
| B13, 14  | HtrA2      | High temperature requirement protein A2                                |
| B15, 16  | LIMK2      | LIM motif-containing protein kinase-2                                  |
| C1, 2    | MPP2       | MAGUK p55 subfamily member 2   |
| C3, 4    | NEB1       | Neurabin-I   |
| C5, 6    | OMP25      | Mitochondrial outer membrane protein 25                                |
| C7, 8    | hCLIM1     | Human 36 kDa carboxyl terminal LIMdomain protein                       |
| C9, 10   | PTPH1      | Protein-tyrosine phosphatase H1  |
| C11, 12  | ZO-2-D1    | Zonula occludens protein 2, PDZ domain #1                              |
| C13, 14  | hPTP1E-D1  | Protein-tyrosine phosphatase 1E, PDZ domain #1                         |
| C15, 16  | hPTP1E-D5  | Protein-tyrosine phosphatase 1E, PDZ domain #5                         |
| D1, 2    | RGS12      | Regulator of G-protein signaling 12                                    |
| D3, 4    | RIL        | Reversion-induced LIM protein  |
| D5, 6    | ZO-1-D3    | Zonula occludens 1 protein, PDZ domain #3                              |
| D7, 8    | ZO-2-D3    | Zonula occludens protein 2, PDZ domain #3                              |
| D9, 10   | GST        | Glutathione-S-Transferase  |

## 14 | TranSignal™ PDZ Domain Arrays

### APPENDIX B: Typical Results, Schematic Diagram & Domain List for PDZ Domain Array II



**Typical results obtained with the TranSignal PDZ Domain Array II.** Class Kv1.4 PDZ ligand from bacterial extract specifically interacts with corresponding PDZ domains. Kv1.4 ligand was expressed as histidine tag fusion protein. Bacterial extract was hybridized with the TranSignal PDZ Domain Array, and the image was acquired using FluorChem™ imager (from Alpha Innotech). Spots with stronger intensities indicate higher binding affinity of the ligand of interest to PDZ Domain(s).

|   | 1           | 2        | 3           | 4           | 5           | 6           | 7           | 8           | 9   | 10  | 11  | 12  | 13  | 14  | 15  | 16  | 17  | 18 |
|---|-------------|----------|-------------|-------------|-------------|-------------|-------------|-------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|----|
| A | KIAA0300-D6 | KIAA0303 | KIAA0316    | KIAA0559    | KIAA0613    | KIAA1719-D7 | KIAA1719-D4 | KIAA1719-D3 |     |     |     |     |     |     |     |     | pos |    |
| B | KIAA1526-D3 | MAST205  | KIAA1849    | PALS1-D8    | PALS1-D9    | PALS1-D1    | PALS1-D2    | PALS1-D5    |     |     |     |     |     |     |     |     | pos |    |
| C | PALS1-D3    | MGC5395  | FLJ23209-D1 | FLJ23209-D2 | KIAA1719-D6 | FLJ00011    | E3KARP-D1   | E3KARP-21   |     |     |     |     |     |     |     |     | pos |    |
| D | NHERF1-D1   | Z01-D1   | Z01-D2      | SDB1-D1     | SDB1-D2     | IL16(2)-D3  | LNX1-D3     | NOS1        |     |     |     |     |     |     |     |     | pos |    |
| E | PDZ-pos     | GST      |             |             |             |             |             |             |     |     |     |     |     |     |     |     | pos |    |
| F | pos         | pos      | pos         | pos         | pos         | pos         | pos         | pos         | pos | pos | pos | pos | pos | pos | pos | pos | pos |    |

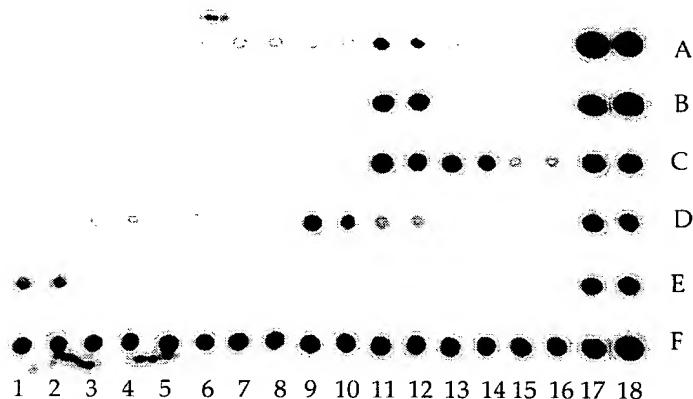
**Schematic diagram of the TranSignal PDZ Domain Array II.** The proteins on the array are spotted in duplicate at 100 ng. Histidine-tagged ligand has been spotted along the bottom (row F) and in duplicate along the right side (column 17, 18) of the membrane. These spots are intended for alignment. Note that the notch is at the top right-hand corner.

## PDZ domain list • TransSignal™ PDZ Domain Array II

| POSITION | PDZ DOMAIN  | ACCESSION  | FULL NAME   |
|----------|-------------|------------|---|
| A1, 2    | KIAA0300-D6 | BAA20760   | KIAA0300 protein, Domain 6  |
| A3, 4    | KIAA0303    | BAA20762   | KIAA0303 protein  |
| A5, 6    | KIAA0316    | BAA20774   | KIAA0316 protein  |
| A7, 8    | KIAA0559    | BAA25485   | KIAA0559 protein  |
| A9, 10   | KIAA0613    | BAA31588   | KIAA0613 protein, Domain 7  |
| A13, 14  | KIAA1719-D4 | BAB21810   | KIAA1719 protein, Domain 4  |
| A15, 16  | KIAA1719-D3 | BAB21810   | KIAA1719 protein, Domain 3  |
| B1, 2    | KIAA1719-D6 | BAB21810   | KIAA1719 protein, Domain 6  |
| B3, 4    | KIAA1526-D3 | BAA96050   | KIAA1526 protein, Domain 3  |
| B5, 6    | MAST205     | BAB40778   | microtubule associated testis specific serine/threonine protein kinase  |
| B7, 8    | PALS1-D8    | AAM28433   | Pals1-associated tight junction protein, Domain 8   |
| B9, 10   | PALS1-D9    | AAM28433   | Pals1-associated tight junction protein, Domain 9   |
| B11, 12  | PALS1-D1    | AAM28433   | Pals1-associated tight junction protein, Domain 1   |
| B13, 14  | PALS1-D2    | AAM28433   | Pals1-associated tight junction protein, Domain 2   |
| B15, 16  | PALS1-D5    | AAM28433   | Pals1-associated tight junction protein, Domain 5   |
| C1, 2    | PALS1-D3    | AAM28433   | Pals1-associated tight junction protein, Domain 3   |
| C3, 4    | MGC5395     | AAH12477   | Similar to hypothetical protein MGC5395   |
| C5, 6    | FLJ23209-D1 | NP_079171  | hypothetical protein FLJ23209, Domain 1   |
| C7, 8    | FLJ23209-D2 | NP_079171  | Hypothetical protein FLJ23209, Domain 2   |
| C9, 10   | KIAA1719-D6 | BAB21810   | KIAA1719 protein, Domain 6  |
| C11, 12  | FLJ00011    | Q9H7Q6     | FLJ00011 protein (Fragment)   |
| C13, 14  | E3KARP-D1   | NP_004776  | solute carrier family 9 (sodium/hydrogen exchanger), 3 regulatory factor 2, Domain 1  |
| C15, 16  | E3KARP-D2   | NP_004776  | solute carrier family 9 (sodium/hydrogen exchanger), 3 regulatory factor 2, Domain 2  |
| D1, 2    | NHERF1-D1   | NP_004243  | solute carrier family 9 (sodium/hydrogen exchanger), Isoform 3 regulatory factor 1, Domain 1  |
| D3, 4    | Q07157      | ZO1-D1     | tight junction protein 1 (zona occludens), Domain 1   |
| D5, 6    | Q07157      | ZO1-D2     | tight junction protein 1 (zona occludens), Domain 2   |
| D7, 8    | NP_005616S  | DB1-D1     | syndecan binding protein (syntenin), melanoma differentiation associated protein-9, Pro-TGF-alpha cytoplasmic domain-interacting protein 18 (TACIP18), Domain 1 |
| D9, 10   | NP_005616S  | DB1-D2     | syndecan binding protein (syntenin), melanoma differentiation associated protein-9, Pro-TGF-alpha cytoplasmic domain-interacting protein 18 (TACIP18), Domain 2 |
| D11, 12  | NP_757366   | IL16(2)-D3 | interleukin 16 isoform 2; lymphocyte chemoattractant factor, Domain 3   |
| D13, 14  | Q8TB21      | LNX1-D3    | Numb-binding protein 1; Ligand of Numb-protein, Domain 3  |
| D15, 16  | NOS1        | NP_000611  | nitric oxide synthase 1 (neuronal), domain 5  |
| E1, 2    | PDZ-pos     |            | PDZ Domain positive control for Kv1.4 ligand  |
| E3, 4    | GST         |            | Glutathione-S-Transferase   |

## 16 | TranSignal™ PDZ Domain Arrays

### APPENDIX C: Typical Results, Schematic Diagram & Domain List for PDZ Domain Array III



*Typical results obtained with the TranSignal PDZ Domain Array III. Class Kv1.4 PDZ ligand from bacterial extract specifically interacts with corresponding PDZ domains. Kv1.4 ligand was expressed as histidine tag fusion protein. Bacterial extract was hybridized with the TranSignal PDZ Domain Array, and the image was acquired using FluorChem™ imager (from Alpha Innotech). Spots with stronger intensities indicate higher binding affinity of the ligand of interest to PDZ Domain(s).*

|   | 1         | 2        | 3        | 4        | 5        | 6         | 7         | 8         | 9         | 10  | 11  | 12  | 13  | 14  | 15  | 16  | 17  | 18 |
|---|-----------|----------|----------|----------|----------|-----------|-----------|-----------|-----------|-----|-----|-----|-----|-----|-----|-----|-----|----|
| A | MAGI3-D2  | MAGI3-D4 | MAGI3-D5 | MAGI3-D6 | BAI1-D2  | BAI1-D3   | BAI1-D4   | BAI1-D1   |           |     |     |     |     |     |     |     | pos |    |
| B | BAI1-D6   | AIP1-D2  | AIP1-D3  | AIP1-D4  | AIP1-D5  | AIP1-D6   | AIP1-D6   | hPTP1E-D2 | hPTP1E-D3 |     |     |     |     |     |     |     | pos |    |
| C | hPTP1E-D4 | PTPN4    | GRIP1-D4 | GRIP1-D3 | GRIP1-D2 | SCRIB1-D1 | SCRIB1-D2 | SCRIB1-D4 |           |     |     |     |     |     |     |     | pos |    |
| D | PARD3-D2  | PARD3-D3 | HARM-D3  | MLL4     | TIP1     | SDB2-D2   |           |           |           |     |     |     |     |     |     |     | pos |    |
| E | PDZ-pos   | GST      |          |          |          |           |           |           |           |     |     |     |     |     |     |     | pos |    |
| F | pos       | pos      | pos      | pos      | pos      | pos       | pos       | pos       | pos       | pos | pos | pos | pos | pos | pos | pos | pos |    |

*Schematic diagram of the TranSignal PDZ Domain Array III. The proteins on the array are spotted in duplicate at 100 ng. Histidine-tagged ligand has been spotted along the bottom (row F) and in duplicate along the right side (column 17, 18) of the membrane. These spots are intended for alignment. Note that the notch is at the top right-hand corner.*

## PDZ domain list • TranSignal™ PDZ Domain Array III

| POSITION | PDZ DOMAIN | ACCESSION | FULL NAME   |
|----------|------------|-----------|---|
| A1, 2    | MAGI3-D2   | NP_690864 | membrane-associated guanylate kinase-related 3, Domain 2  |
| A3, 4    | MAGI3-D4   | NP_690864 | membrane-associated guanylate kinase-related 3, Domain 4  |
| A5, 6    | MAGI3-D5   | NP_690864 | membrane-associated guanylate kinase-related 3, Domain 5  |
| A7, 8    | MAGI3-D6   | NP_690864 | membrane-associated guanylate kinase-related 3, Domain 6  |
| A9, 10   | BAI1-D2    | NP_004733 | brain-specific angiogenesis inhibitor-associated protein 1; WW domain-containing protein 3; atrophin-1 interacting protein 3, Domain 2  |
| A11, 12  | BAI1-D3    | NP_004733 | brain-specific angiogenesis inhibitor-associated protein 1; WW domain-containing protein 3; atrophin-1 interacting protein 3, Domain 3  |
| A13, 14  | BAI1-D4    | NP_004733 | brain-specific angiogenesis inhibitor-associated protein 1; WW domain-containing protein 3; atrophin-1 interacting protein 3, Domain 4  |
| A15, 16  | BAI1-D1    | NP_004733 | brain-specific angiogenesis inhibitor-associated protein 1; WW domain-containing protein 3; atrophin-1 interacting protein 3, Domain 1  |
| B1, 2    | BAI1-D6    | NP_004733 | brain-specific angiogenesis inhibitor-associated protein 1; WW domain-containing protein 3; atrophin-1 interacting protein 3, Domain 6  |
| B3, 4    | AIP1-D2    | NP_036433 | atrophin-1 interacting protein 1, Domain 2  |
| B5, 6    | AIP1-D3    | NP_036433 | atrophin-1 interacting protein 1, Domain 3  |
| B7, 8    | AIP1-D4    | NP_036433 | atrophin-1 interacting protein 1, Domain 4  |
| B9, 10   | AIP1-D5    | NP_036433 | atrophin-1 interacting protein 1, Domain 5  |
| B11, 12  | AIP1-D6    | NP_036433 | atrophin-1 interacting protein 1, Domain 6  |
| B13, 14  | hPTP1E-D2  | NP_542416 | protein tyrosine phosphatase, non-receptor type 13 isoform 4; protein-tyrosine phosphatase PTPL1; protein tyrosine phosphatase 1E; Fas-associated phosphatase-1; protein-tyrosine phosphatase 1, Fas-associated APO-1/CD95 (Fas)-associated phosphatase, Domain 2 |
| B15, 16  | hPTP1E-D3  | NP_542416 | protein tyrosine phosphatase, non-receptor type 13 isoform 4; protein-tyrosine phosphatase PTPL1; protein tyrosine phosphatase 1E; Fas-associated phosphatase-1; protein-tyrosine phosphatase 1, Fas-associated APO-1/CD95 (Fas)-associated phosphatase, Domain 3 |
| C1, 2    | hPTP1E-D4  | NP_542416 | protein tyrosine phosphatase, non-receptor type 13 isoform 4; protein-tyrosine phosphatase PTPL1; protein tyrosine phosphatase 1E; Fas-associated phosphatase-1; protein-tyrosine phosphatase 1, Fas-associated APO-1/CD95 (Fas)-associated phosphatase, Domain 4 |
| C3, 4    | JTPN4      | NP_002821 | protein tyrosine phosphatase, non-receptor type 4; megakaryocyte phosphatase; PTPase-MEG1   |
| C5, 6    | GRIP1-D4   | CAB39895  | GRIP1 protein, Domain 4   |
| C7, 8    | GRIP1-D3   | CAB39895  | GRIP1 protein, Domain 3   |
| C9, 10   | GRIP1-D2   | CAB39895  | GRIP1 protein, Domain 2   |
| C11, 12  | SCRIB1-D1  | NP_056171 | Scribble, Domain 1  |
| C13, 14  | SCRIB1-D2  | NP_056171 | Scribble, Domain 2  |
| C15, 16  | SCRIB1-D4  | NP_056171 | Scribble, Domain 4  |

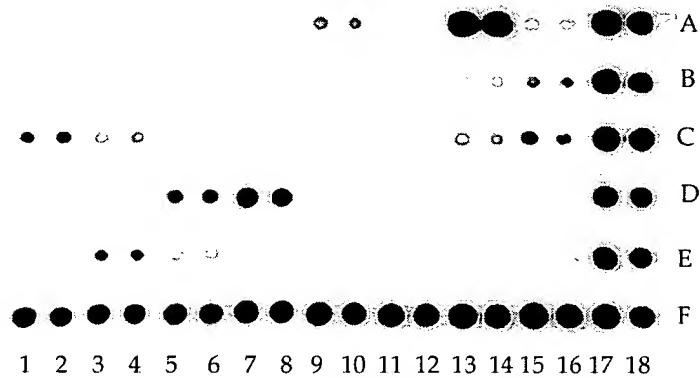
## 18 | TranSignal™ PDZ Domain Arrays

| POSITION | PDZ DOMAIN | ACCESSION | FULL NAME   |
|----------|------------|-----------|---|
| D1, 2    | PARD3-D2   | NP_062565 | partitioning-defective protein 3 homolog; atypical PKC isotype-specific interacting protein, Domain 2 |
| D3, 4    | PARD3-D3   | NP_062565 | partitioning-defective protein 3 homolog; atypical PKC isotype-specific interacting protein, Domain 3 |
| D5, 6    | HARM-D3    | NP_005700 | harmonin; PDZ-73 protein; antigen NY-CO-38, Domain 3  |
| D7, 8    | MLL4       | NP_005927 | myeloid/lymphoid or mixed-lineage leukemia, translocated to, 4  |
| D9, 10   | TIP1       | NP_055419 | Tax interaction protein 1   |
| D11, 12  | SDB2-D2    | NP_056500 | syntenin-2beta; syntenin-2; similar to syndecan binding protein, Domain 2                             |
| E1, 2    | PDZ-pos    |           | PDZ Domain positive control for Kv1.4 ligand  |
| E3, 4    | GST only   |           | Glutathione-S-Transferase   |

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## APPENDIX D: Typical Results, Schematic Diagram & Domain List for PDZ Domain Array IV



*Typical results obtained with the TransSignal PDZ Domain Array IV. Class Kv1.4 PDZ ligand from bacterial extract specifically interacts with corresponding PDZ domains. Kv1.4 ligand was expressed as histidine tag fusion protein. Bacterial extract was hybridized with the TransSignal PDZ Domain Array, and the image was acquired using FluorChem™ imager (from Alpha Innotech). Spots with stronger intensities indicate higher binding affinity of the ligand of interest to PDZ Domain(s).*

|   | 1        | 2         | 3        | 4        | 5         | 6        | 7       | 8       | 9   | 10  | 11  | 12  | 13  | 14  | 15  | 16  | 17  | 18 |
|---|----------|-----------|----------|----------|-----------|----------|---------|---------|-----|-----|-----|-----|-----|-----|-----|-----|-----|----|
| A | MUPPI-D6 | MUPPI-D12 | MUPPI-D2 | MUPPI-D3 | MUPPI-D13 | MUPPI-D1 | DLG3-D2 | DLG3-D3 |     |     |     |     |     |     |     |     | pos |    |
| B | DLG5-D1  | DLG5-D4   | DLG5-D3  | DLG5-D2  | DLG2-D3   | PAR68    | UK1     | LAMP    |     |     |     |     |     |     |     |     | pos |    |
| C | RIL      | A2LM      | TIAM1    | LIN7C    | LIN7B     | LIN7A    | GEF11   | GEF12   |     |     |     |     |     |     |     |     | pos |    |
| D | PDZK1-D1 | PDZK1-D2  | SNB1     | SNA1     | SHK1      | MPP6     | PIST    | GEF2    |     |     |     |     |     |     |     |     | pos |    |
| E | RIN2     | PDZ-pos   | GSI      |          |           |          |         |         |     |     |     |     |     |     |     |     | pos |    |
| F | pos      | pos       | pos      | pos      | pos       | pos      | pos     | pos     | pos | pos | pos | pos | pos | pos | pos | pos | pos |    |

*Schematic diagram of the TransSignal PDZ Domain Array IV. The proteins on the array are spotted in duplicate at 100 ng. Histidine-tagged ligand has been spotted along the bottom (row F) and in duplicate along the right side (column 17, 18) of the membrane. These spots are intended for alignment. Note that the notch is at the top right-hand corner.*

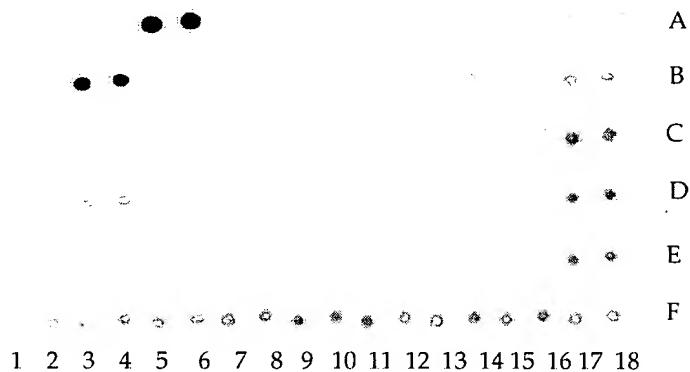
## 20 | TranSignal™ PDZ Domain Arrays

### PDZ domain list • TranSignal™ PDZ Domain Array IV

| POSITION | PDZ DOMAIN     | ACCESSION | FULL NAME  |
|----------|----------------|-----------|--|
| A1, 2    | MUPP1-D6       | NP_003820 | multiple PDZ domain protein, Domain 6  |
| A3, 4    | MUPP1-D12      | NP_003820 | multiple PDZ domain protein, Domain 12   |
| A5, 6    | MUPP1-D2       | NP_003820 | multiple PDZ domain protein, Domain 2  |
| A7, 8    | MUPP1-D3       | NP_003820 | multiple PDZ domain protein, Domain 3  |
| A9, 10   | MUPP1-D13      | NP_003820 | multiple PDZ domain protein, Domain 13   |
| A11, 12  | MUPP1-D1       | NP_003820 | multiple PDZ domain protein, Domain 1  |
| A13, 14  | DLG3-D2        | NP_066943 | synapse-associated protein 102; neuroendocrine-dlg; discs large homolog 3, Domain 2  |
| A15, 16  | DLG3-D3        | NP_066943 | synapse-associated protein 102; neuroendocrine-dlg; discs large homolog 3, Domain 3  |
| B1, 2    | DLG5-D1        | NP_004738 | discs, large homolog 5, Domain 1   |
| B3, 4    | DLG5-D4        | NP_004738 | discs, large homolog 5, Domain 4   |
| B5, 6    | DLG5-D3        | NP_004738 | discs, large homolog 5, Domain 3   |
| B7, 8    | DLG5-D2        | NP_004738 | discs, large homolog 5, Domain 2   |
| B9, 10   | DLG2-D3        | NP_001355 | Channel associated protein of synapse-110 (Chapsyn-110), Domain 3  |
| B11, 12  | PAR6B          | BAB40756  | Partitioning defective-6 homolog beta  |
|          |                |           | Domain 1   |
| B13, 14  | LIK1           | NP_002305 | LIM domain kinase 1 isoform 1; LIM motif-containing protein kinase   |
| B15, 16  | LOMP           | NP_005349 | LIM domain only 7 isoform a; KIAA0858 protein  |
| C1, 2    | RIL            | NP_003678 | LIM protein RIL (Reversion-induced LIM protein)  |
| C3, 4    | A2LIM          | NP_055291 | alpha-actinin-2-associated LIM protein; enigma homolog   |
| C5, 6    | TIAM1          | NP_003244 | T-cell lymphoma invasion and metastasis 1  |
| C7, 8    | LIN7C          | NP_060832 | Lin-7 homolog C  |
| C9, 10   | LIN7B          | NP_071448 | Lin-7 homolog B  |
| C11, 12  | LIN7A          | NP_004655 | Lin-7 homolog A  |
| C13, 14  | GEF11          | NP_055599 | Rho guanine exchange factor (GEF) 11; glutamate transporter EAAT4-associated protein 48; KIAA0380 protein  |
| C15, 16  | GEF12          | NP_056128 | Rho guanine exchange factor (GEF) 12; leukemia-associated GEF; similar to mouse Lsc oncogene   |
| D1, 2    | PDZK1-D1       | NP_002605 | PDZ domain containing 1, Domain 1  |
| D3, 4    | PDZK1-D2       | NP_002605 | PDZ domain containing 1, Domain 2  |
| D5, 6    | SNB1           | NP_066301 | Beta-1-syntrophin; tax interaction protein 43; dystrophin-associated protein A1, 59kD, basic component 1   |
| D7, 8    | SNA1           | NP_003089 | acidic alpha 1 syntrophin; dystrophin-associated protein A1, 59kD, acidic component; pro-TGF-alpha cytoplasmic domain-interacting protein 1  |
| D9, 10   | SHK1           | NP_057232 | somatostatin receptor-interacting protein; SH3 and multiple ankyrin repeat domains 1   |
| D11, 12  | MPP6           | NP_057531 | membrane protein, palmitoylated 6; protein associated with Lin7 2; VELI-associated MACUK 1; MAGUK protein p55T   |
| D13, 14  | PIST           | NP_065132 | Golgi associated and coiled-coil motif containing protein; CFTR-associated PDZ/coiled-coil domain binding partner for the rho-family GTPase TC10; fused in glioblastoma; Golgi associated PDZ and coiled-coil motif containing protein |
| D15, 16  | GEF2           | NP_057424 | Rap guanine nucleotide exchange factor; PDZ domain-containing guanine nucleotide exchange factor I   |
| E1, 2    | RIM2           | NP_055492 | regulating synaptic membrane exocytosis 2; RAB3 interacting protein 3; KIAA0751 protein  |
| E3, 4    | PDZ-pos<br>GST |           | PDZ Domain positive control for Kv1.4 ligand<br>Glutathione-S-Transferase  |

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## APPENDIX E: Biotin-Conjugated Peptide with PDZ Domain Arrays



*Binding of a biotin-conjugated PDZ ligand peptide on PDZ Domain Array II.* Membrane was incubated with Blocking Buffer for 1 h, rinsed once with 1X Wash Buffer and probed with Peptide Probe Mix for 1 h (Peptide Probe Mix: mix 15  $\mu$ l of 0.1 mM biotin-conjugated peptide with 1 mg/ml Streptavidin-HRP; incubate with gentle rocking for 30 min at 4°C; and add to 12 ml of 1X Blocking Buffer). Membrane was washed three times with 1X Wash Buffer after incubation with the peptide. Chemiluminescent signal was detected as described in Section 8 and the image was acquired using FluorChem™ imager (from Alpha Innotech).

## **22 | TranSignal™ PDZ Domain Arrays**

### **NOTES:**

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